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Establishment of Reference Ranges for Thyrotropin, Triiodothyronine, Thyroxine and Free Thyroxine in Neonates, Infants, Children and Adolescents

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Summary: Thyrotropin, triiodothyronine, thyroxine and free thyroxine were determined in the sera of 714 euthyreotic neonates, infants, children and adolescents (age range 5 days to 18 years), using the IMx from Abbott Laboratories. Thyrotropin, triiodothyronine and free thyroxine were determined with microparticle enzyme immunoassays (MEIA). Thyroxine was determined with a fluorescence polarization immunoassay (FPIA). The proband collective was divided into 9 age groups, and each age group into males and females. In accordance with the recommendations of the International Federation of Clinical Chemistry, the 95% scatter range was taken as the reference range. In the different age groups, the ranges of some hormones showed significant differences between males and females. When no significant difference existed between the sexes, the results for males and females were evaluated statistically as a single group. Only a few reference groups showed normal *Gauss*ian distributions. Therefore, in addition to the 50th percentile, the 2.5th and 97.5th percentiles were also calculated for all reference groups. Minimal and maximal values were also determined. The U-test of *Mann & Whitney* was used to test for significant differences between individual reference groups, and groups showing no significant differences were combined. The corresponding references ranges were then calculated.

Introduction

The concentrations of thyrotropin and thyroid hormones in serum are indices of the degree of hyper- or hypoactivity of the thyroid gland. In known disturbances of thyroid function, the concentrations of thyroid hormones in serum serve as control values for the efficacy of thyrostatic treatment, or of substitution therapy with thyroid hormones. Serum thyroxine displays strong protein binding, so that its concentration is easily affected by the concentration or binding capacity of its binding proteins. The concentration of free thyroxine, i. e. the only part of the thyroxine that is metabolically active, is diagnostically more useful than the total thyroxine concentration. However, there is still no reliable method for the determination of free thyroxine in serum under all conditions, because of possible interference by albumin, free fatty acids and thyroxine-binding globulin (thyropexin) 1–5).

The aim of the investigation was:

1) to establish reference ranges for thyrotropin, triiodothyronine (T_3), thyroxine (T_4) and free thyroxine (fT_4) in healthy neonates, infants, children and adolescents;

2) to test for significant sex differences in the studied quantities within the age groups;

3) to test for significant differences in the studied quantities between the age groups.

Materials and Methods

Thyrotropin, triiodothyronine, thyroxine and free thyroxine were determined in the sera of 714 healthy neonates, infants, children and adolescents in the age range 5 days to 18 years. The age composition of the proband collective is summarized in table 1. Individuals were included or excluded according to the exclusion criteria of *Witt & Trendelenburg* (6), which permit the assembly of a reliable random reference sample at a justifiable expense. Only those neonates with a birth weight between 2500 and 4000 g and a full term gestation time between 37 and 40 weeks were admitted to the 5-day-old age group. Neonates with hyperbilirubinaemia were excluded, as well as those born to mothers with acute or chronic illnesses. All probands were free from any signs of hypo- or hyperthyreosis.

Tab. 1. Age structure of the proband collective for the determination of reference ranges for thyrotropin, triiodothyronine, thyroxine and free thyroxine in neonates, infants, children and adolescents

Group	Age	n			
		Thyrotropin	T_3	T_4	fT_4
1 ♂	5 days	71	71	70	56
1 ♀	5 days	73	70	71	53
1	5 days	143	141	141	109
2 ♂	2–12 months	11	15	13	15
2 ♀	2–12 months	12	13	12	12
2	2–12 months	23	28	25	27
3 ♂	2– 3 years	18	18	17	16
3 ♀	2– 3 years	19	18	19	19
3	2– 3 years	37	36	36	35
4 ♂	4– 6 years	43	43	43	43
4 ♀	4– 6 years	26	25	26	22
4	4– 6 years	69	68	69	65
5 ♂	7– 9 years	46	46	46	45
5 ♀	7– 9 years	41	41	41	35
5	7– 9 years	87	87	87	84
6 ♂	10–11 years	45	45	45	44
6 ♀	10–11 years	55	54	55	53
6	10–11 years	100	99	100	97
7 ♂	12–13 years	46	46	45	45
7 ♀	12–13 years	46	46	47	45
7	12–13 years	92	92	92	90
8 ♂	14–15 years	41	41	40	39
8 ♀	14–15 years	37	38	37	38
8	14–15 years	78	79	77	77
9 ♂	16–18 years	38	38	38	37
9 ♀	16–18 years	37	38	35	37
9	16–18 years	75	76	73	74

Test material

About 2 ml of blood were taken, with the informed consent of the parents and the consent of the Ethics Commission of the Medical University Erfurt, between 08.00 and 10.00 am, from an arm or skull vein, using safety monovettes from the firm of Sarstedt, Nümbrecht. Blood samples were centrifuged immediately for 5 min at 3000 min^{-1}. The serum was removed with a pipette, then frozen at −22 °C until analysed.

Method

Thyrotropin, triiodothyronine, thyroxine and free thyroxine were all determined with the aid of the IMx from Abbott laboratories. Microparticle enzyme immunoassays were used for thyrotropin, T_3 and fT_4. The thyrotropin standard 80/588 from the WHO was used as a calibrator for the thyrotropin determination. T_4 was determined with the IMx T_4 test from Abbott Laboratories, which is a fluorescence polarization immunoassay (FPIA). The lower detection limits and cross reactivities of the tests are shown in table 2.

Tab. 2. Data provided by the manufacturer on the lower detection limits and cross reactivities of the tests used on the IMx (the concentration of each test substance used is shown in brackets). The lower detection limit is defined as the lowest concentration that can be differentiated from 0, i.e. the value that is two standard deviations higher than the zero calibrator.

Test	Lower detection limit	Cross reactivity
IMx hTSH Ultrasensitive	0.03 mU/l	with lutropin (3000 U/l): $3.3 \cdot 10^{-6}$ with follitropin (3000 U/l): $5.1 \cdot 10^{-4}$ with human chorionic gonadotropin ($1.7 \cdot 10^6$ U/l): $2.2 \cdot 10^{-8}$
IMx Total T_3	0.15 µg/l	with T_4 (1100 µg/l): none
IMx T_4	10.0 µg/l	with T_3: < 10%
IMx free T_4	4.00 ng/l	with T_3: < 0.5%

Quality control

For the determination of precision from day to day, standards of low, intermediate and high concentration (from Abbott and from Rolf Greiner Biochemica, Flacht) were included intermittently in each series. As a measure of the relative methodical error, the arithmetic mean ($\bar{x}$), standard deviation (s) and the variation coefficient (CV) were calculated from the individual results of these control series. Precision is series was monitored once, using the control sera, Serodos and Serodos Plus, from Greiner. Again, the arithmetic mean ($\bar{x}$), standard deviation (s) and the variation coefficient (CV) were calculated from the individual results.

Statistical evaluation of the results

The results were first presented as separate histograms for each age group and for each sex. Obvious outliers were eliminated. The type of distribution was determined with the *Kolmogorov-Smirnov* test. If the resulting probability error was below the stated value of $\alpha = 0.05$, the distribution was assumed to be normal. If the distribution was not normal, the 2.5th, 50th and 97.5th percentiles were determined for that age group (7).

Each age group was tested for a significant difference between the sexes, using the U-test of *Mann & Whitney*. In the absence of a significant sex difference, males and females were subsequently treated as a single group. The significance of differences between age groups was also tested with the U-test of *Mann & Whitney*.

The degree of any linear relationship between age and the measured quantities was determined by calculation of the correlation coefficient, r.

Results

Thyrotropin

Thyrotropin was determined in the sera of 704 euthyreotic probands (359 boys, 345 girls). Figure 1 gives an overview of the results for all groups before they were tested for significance. Although 12 to 13-year old girls appear to constitute a special group, they were not significantly different from the 10 to 11-year old girls ($p = 0.6379$); they were therefore combined with the latter to form a single group. Similarly, other groups were combined, if they displayed no significant difference. For each group combination made in this way the median and the reference range for serum thyrotropin were recalculated; the results are summarized in table 3.

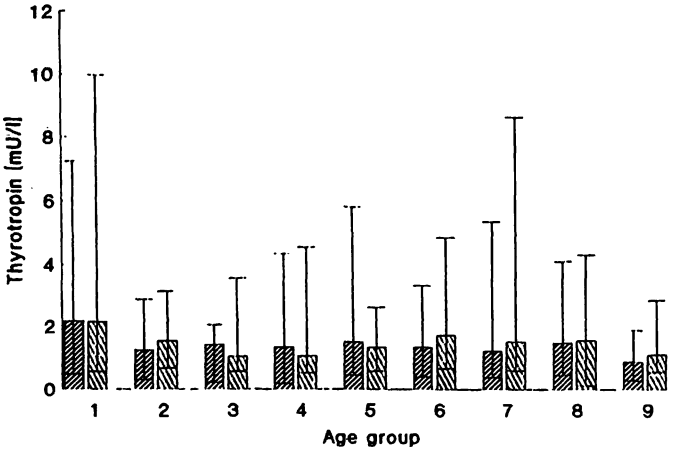

Fig. 1. Histograms of the 50th percentiles (males ▨, females ▧) and the 95% intervals of the serum concentrations of thyrotropin (mU/l) in the age groups 1–9 (see table 1).

Triiodothyronine

Triiodothyronine was determined in the sera of 706 euthyreotic probands (363 boys, 343 girls). Figure 2 gives an overview of the results for all groups before they were tested for significance. Although no significant differences were found between the results for 5-day-old neonates, 14–15-year old girls and 16–18-year old boys, these groups were treated separately. The new groupings formed after testing for significance, together with their newly calculated median values and reference ranges are shown in table 4.

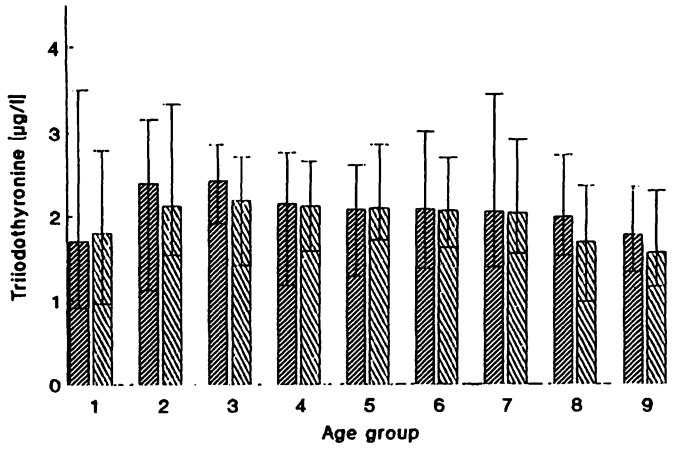

Fig. 2. Histograms of the 50th percentiles (males ▨, females ▧) and the 95% intervals of the serum concentrations of T_3 (µg/l) in the age groups 1–9 (see table 1).

Thyroxine

Thyroxine was determined in the sera of 700 euthyreotic probands (357 boys, 343 girls). Figure 3 gives an overview of the results for all groups before they were tested for significance. New grouping formed after testing for significance, together with their newly calculated median values and reference ranges are shown in table 5.

Tab. 3. Summary of results for the serum concentrations of thyrotropin in euthyreotic neonates, infants, children and adolescents, showing 50th percentiles, 95% intervals and the minimal and maximal values. Values are given in mU/l.

Age	Sex	n	Median (50th percentile)	Reference range (95% scatter range: 2.5th–97.5th percentiles)	Minimum	Maximum
5 days	♂/♀	143	2.17	0.51–7.90	0.38	10.6
2 months–9 years 10–13 years 14–15 years	♂/♀ ♂ ♂/♀	385	1.33	0.41–3.45	0.12	6.35
10–13 years	♀	101	1.58	0.64–4.76	0.59	9.47
16–18 years	♂	38	0.88	0.28–1.88	0.28	1.88
16–18 years	♀	37	1.10	0.53–2.85	0.53	2.85

Tab. 4. Summary of results for the serum concentrations of T_3 in euthyreotic neonates, infants, children and adolescents, showing 50th percentiles, 95% intervals and the minimal and maximal values. Values are given in μg/l.

Age	Sex	n	Median (50th percentile)	Reference range (95% scatter range: 2.5th–97.5th percentiles)	Minimum	Maximum
5 days	♂/♀	141	1.76	0.99–3.04	0.71	5.21
2 months–3 years	♂/♀	64	2.29	1.30–3.23	1.12	3.34
4–13 years	♂/♀	346	2.09	1.50–2.70	1.18	5.50
14–15 years	♂	41	1.99	1.53–2.73	1.53	2.73
14–15 years 16–18 years	♀ ♂	76	1.77	1.16–2.35	0.98	2.36
16–18 years	♀	38	1.56	1.16–2.30	1.16	3.20

Tab. 5. Summary of results for the serum concentrations of T_4 in euthyreotic neonates, infants, children and adolescents, showing 50th percentiles, 95% intervals and the minimal and maximal values. Values are given in μg/l.

Age	Sex	n	Median (50th percentile)	Reference range (95% scatter range: 2.5th–97.5th percentiles)	Minimum	Maximum
5 days	♂/♀	141	161	81.3–233	57.8	140
2–12 months 2– 3 years	♂/♀ ♀	44	95.5	66.5–158	65.1	158
2– 3 years 4– 6 years 10–13 years	♂ ♂/♀ ♂/♀	278	79.6	55.1–113	46.8	146
7– 9 years	♂/♀	87	88.2	54.3–130	54.0	139
14–18 years	♂/♀	150	70.6	42.3– 99.1	34.4	140

Free thyroxine

Free thyroxine was determined in the sera of 658 healthy probands (340 boys, 318 girls). Figure 4 gives an overview of the results for all groups before they were tested for significance. There were no significant sex differences in any age group. A weak, significant difference was found between age groups 7 and 8, but no significant differences were detectable between age groups 7 and 9, or between age groups 8 and 9. These three groups also displayed very similar median values and reference ranges; they were therefore combined, and the median value and reference range were recalculated. The new groupings formed after testing for significance are shown in table 6.

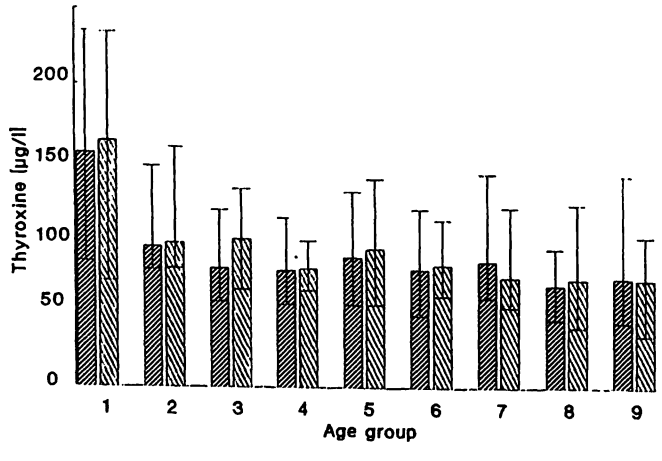

Fig. 3. Histograms of the 50th percentiles (males ▨, females ▧) and the 95% intervals of the serum concentrations of T_4 (μg/l) in the age groups 1–9 (see table 1).

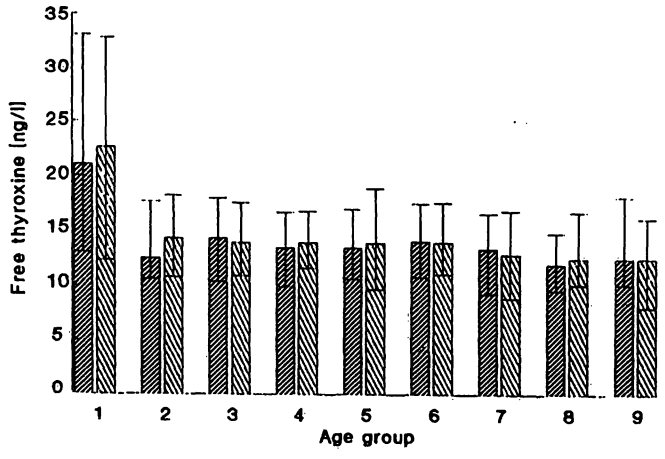

Fig. 4. Histograms of the 50th percentiles (males ▨, females ▧) and the 95% intervals of the serum concentrations of fT_4 (ng/l) in the age groups 1–9 (see table 1).

Tab. 6. Summary of results for the serum concentrations of fT_4 in euthyreotic neonates, infants, children and adolescents, showing 50th percentiles, 95% intervals and the minimal and maximal values. Values are given in ng/l.

Age	Sex	n	Median (50th percentile)	Reference range (95% scatter range: 2.5th–97.5th percentiles)	Minimum	Maximum
5 days	♂/♀	109	21.9	13.0–32.9	11.9	33.4
2 months–11 years	♂/♀	308	12.8	10.7–17.5	9.7	18.8
12–18 years	♂/♀	241	12.5	9.2–15.9	8.00	18.0

Correlation analysis

Correlations with proband age were sought for the serum concentrations of thyrotropin, T_3, T_4 and fT_4. The serum concentrations of all four hormones decreased significantly ($p < 0.001$) with the increasing age (in months) of the studied children: thyrotropin ($r = -0.1989$), triiodothyronine ($r = -0.1259$), thyroxine ($r = -0.6540$) and free thyroxine ($r = -0.5999$).

Quality control

Results of the quality control are shown in tables 7 and 8. With one exception, the variation coefficients within series and between series were less than 10%.

Tab. 7. Results for the control of precision from day to day

	Control serum	n	$\bar{x}$	s	CV (%)
Thyrotropin (mU/l)	Serodos	19	1.00	0.08	8.00
	Serodos Plus	31	14.0	0.76	5.44
	Abbott M	56	5.84	0.26	4.45
	Abbott H	22	48.0	4.24	8.82
T_3 (µg/l)	Abbott L	22	0.73	0.08	10.9
	Abbott M	32	1.50	0.14	9.33
	Abbott H	22	3.75	0.21	5.60
T_4 (µg/l)	Abbott L	26	48.0	4.40	9.17
	Abbott M	32	81.3	5.10	6.27
	Abbott H	24	151	8.40	5.56
fT_4 (ng/l)	Abbott L	13	7.10	0.40	5.63
	Abbott M	16	12.5	0.60	4.80
	Abbott H	10	33.7	1.40	4.15

Tab. 8. Results for the control of precision in series

	Control serum	n	$\bar{x}$	s	CV (%)
Thyrotropin (mU/l)	Serodos	22	1.05	0.04	3.72
	Serodos Plus	22	34.4	1.07	3.11
T_3 (µg/l)	Serodos	23	4.09	0.17	4.15
	Serodos Plus	23	5.89	0.23	3.86
T_4 (µg/l)	Serodos	20	153	5.30	3.49
	Serodos Plus	20	190	4.5	2.36
fT4 (ng/l)	Serodos	23	39.7	1.60	3.99
	Serodos Plus	23	45.6	1.90	4.20

Discussion

In addition to the anamnesis and the interpretation of clinical symptoms, the determination of hormone concentrations in serum is extremely important in the diagnosis of pathological endocrine conditions. To enable the correct evaluation of the serum concentration of a hormone, reference ranges must be established.

In the present study, reference ranges were determined for the serum concentrations of thyrotropin, triiodothyronine, thyroxine and free thyroxine, using the IMx from Abbott Laboratories. The analytical methods were microparticle enzyme immunoassays (MEIA) and a fluorescence polarization enzyme immunoassay (FPIA). Both methods produce rapid results, require only a small sample volume, and do not employ radioactive isotopes. Reference ranges for children, using these methods, have not been reported in the literature. In fact, for most modern immunometric assays, the majority of reported reference ranges are for adults. Due to the processes of child growth and development, however, clinical chemical quantities may differ in children and adults. Such differences must be taken into account in the evaluation of reference ranges.

In accordance with the recommendations of the International Federation of Clinical Chemistry, the 95% scatter range (i.e. the range between the 2.5th and 97.5th percentiles) was taken as the reference range (7).

Each of the four measured quantities in each of the nine age groups shown in table 1 were tested for sex-specific differences, using the U-test of *Mann & Whitney*. Age groups showing no significant differences between male and female were treated as a single reference group. The distribution in each group was investigated with the aid of the *Kolmogorov-Smirnov* test. In the majority of the age groups, the reference values did not show a normal distribution. The reference range was therefore established by reporting the median value, together with the 2.5th and 97.5th percentiles. The U-test of *Mann & Whitney* was also

used to test for significant differences between the serum hormone concentrations of different age groups. Age groups showing no significant differences with respect to a particular hormone, were generally combined, and the median value and percentiles recalculated for that hormone.

To facilitate comparison of the present results with those from the literature, the latter are presented in tables 9 to 12.

The reference ranges for the serum concentrations of thyrotropin, triiodothyronine, thyroxine and free thyroxine reported in the present study are not comparable with the normal ranges reported by other authors, which are shown in tables 9 to 12. This is because:

1) different methods were used;

2) the age groups were different;

3) the number of probands in each reference group was very small;

4) with the exception of *Borkenstein* et al. (8) and *Struckmeyer & Haid* (9), the authors gave no data on the type of distribution of the reference values;

5) no details are given about the international reference preparation (WHO standard) that was used for calibration of the results.

Liappis et al. (10–12) gave the reference range as $\bar{x} \pm 2\,s$, and tested for significance between age groups with *Student*'s t-test. This procedure is valid only for normal *Gauss*ian distributions.

Tab. 9. Reference ranges reported in the literature for the serum concentration of thyrotropin.

Author	Method	Age group	No. of probands	Type of distribution and chosen scatter range	Reference range
Liappis et al., 1988 (11)	LEIA (mU/l)	1–2 days	34	No clear data	9.53 ± 6.79 7.46 to 23.11
		3–30 days	67		1.98 ± 1.01 1.71 to 4.00
		1–12 months	56	$\bar{x} \pm 1s$ Median value 95% Scatter range	1.75 ± 0.95 1.56 to 3.65
		1–7 years	68		1.61 ± 0.56 1.48 0.50 – 0.73
		7–13 years	77		1.66 ± 0.65 1.53 0.36 – 2.96
		13–18 years	41		1.61 ± 0.79 1.39 0.03 – 3.19
Liappis et al., 1991 (12)	LEIA (mU/l)	1–2 days	15	No clear data	8.17 ± 5.44 6.10 to 19.05
		3–30 days	46		1.96 ± 1.23 1.55 to 4.42
		1–12 months	62	$\bar{x} \pm 1s$ Median value 95% Scatter range	1.65 ± 0.76 1.58 0.13 – 3.17
		1–7 years	50		1.47 ± 0.63 1.44 0.21 – 2.73
		7–13 years	52		1.42 ± 0.52 1.32 0.38 – 2.46
		13–18 years	48		1.36 ± 0.56 1.33 0.24 – 2.48

Tab. 9. Continued

Author	Method	Age group	No. of probands	Type of distribution and chosen scatter range	Reference range
Wiedemann et al., 1992 (14)	MEIA (mU/l) IRP 80/558	5 days	1712	No normal distribution Median value 95% Scatter range (2.5th–9.5th percentile)	1.9 (0.40–9.05)
Schönberg & Klett, 1980 in *Schönberg*, 1990 (13)	RIA (mU/l)	0.5– 1 month 1.5– 2 months 2.5– 3 months 3.5– 4 months 4.5– 5 months 5.5– 7 months 8.0–12 months	25 19 12 11 11 7 7	No data Median value ± 1 standard deviation	0.93 ± 0.98 1.34 ± 1.16 0.99 ± 0.83 1.08 ± 0.96 0.75 ± 0.86 0.45 ± 0.74 0.77 ± 0.65
Borkenstein et al., 1980 (8)	RIA (mU/l)	2 months–14 years	182	No normal distribution Median value ± 1 standard deviation	2.25 ± 1.96 (0.00–6.16)*
Struckmeyer & Haid, 1986 (9)	RIA (mU/l) MRC 68/30	♂ 4 days 2 weeks–1 year 1– 6 years 6–10 years 10–16 years		No clear data Median value 90% Scatter range (5% to 95% quantile)	4.60 (1.89–9.08) 2.40 (0.24–5.31) 1.80 (0.80–6.44) 1.95 (1.05–3.51) 2.65 (0.14–6.05)
		♀ 4 days 2 weeks–1 year 1– 6 years 6–10 years 10–16 years			4.60 (1.89–9.08) 1.80 (0.30–5.30) 1.75 (0.20–6.05) 1.85 (1.00–3.36) 3.00 (0.57–5.83)

* The reference range was taken as the interval between ($\bar{x}$ − 1s) and ($\bar{x}$ + 2s). Accordingly, the lower limit can be calculated to be 0.29 mU/l, but the original paper quotes a lower limit of 0.0 mU/l.

Tab. 10. Reference ranges reported in the literature for the serum concentration of tiiodothyronine

Author	Method	Age group	No. of probands	Type of distribution and chosen scatter range	Reference range
Liappis et al., 1988 (11)	LEIA (µg/l)	1–2 days	34	No clear data	1.77 ± 0.57 1.71 0.63 – 2.91
		3–30 days	67		1.44 ± 0.34 1.38 0.76 – 2.12
		1–12 months	56	$\bar{x}$ ± 1s Median value 95% Scatter range	1.56 ± 0.30 1.53 0.96 – 2.16
		1–7 years	68		1.39 ± 0.25 1.35 0.89 – 1.89
		7–13 years	77		1.30 ± 0.22 1.28 0.86 – 1.74
		13–18 years	41		1.25 ± 0.31 1.19 0.63 – 1.87

Continued p. 284

Tab. 10. Continued

Author	Method	Age group	No. of probands	Type of distribution and chosen scatter range	Reference range
Liappis et al., 1991 (12)	LEIA (µg/l)	1–2 days	15	No clear data	1.71 ± 0.45 1.71 0.81 – 2.61
		3–30 days	46		1.38 ± 0.32 1.40 0.74 – 2.02
		1–12 months	62	$\bar{x}$ ± 1s Median value 95% Scatter range	1.74 ± 0.30 1.73 1.14 – 2.34
		1–7 years	50		1.61 ± 0.20 1.65 1.21 – 2.01
		7–13 years	52		1.55 ± 0.21 1.54 1.13 – 1.97
		13–18 years	48		1.43 ± 0.20 1.38 1.03 – 1.83
Schönberg & Klett, 1980 in *Schönberg*, 1990 (13)	RIA (µg/l)	0.5– 1 month 1.5– 2 months 2.5– 3 months 3.5– 4 months 4.5– 5 months 5.5– 7 months 8.0–12 months	25 19 12 11 11 7 7	No data Mean value ± 1s	1.98 ± 0.52 1.84 ± 0.54 2.23 ± 0.47 1.98 ± 0.46 1.99 ± 0.35 2.08 ± 0.58 1.81 ± 0.39
Borkenstein et al., 1980 (8)	RIA (µg/l)	2 months–14 years	182	No normal distribution Mean value ± 1s	1.07 ± 0.34 (0.74 – 1.74)*
Struckmeyer & Haid, 1986 (9)	RIA (µg/l)	♂ 2 weeks 3 weeks–1 year 1– 6 years 6–10 years 10–16 years		No clear data No normal distribution Median value 90% Scatter range (5% to 95% quantile)	1.61 (0.94–2.13) 1.72 (1.22–2.48) 1.65 (1.18–2.22) 1.65 (1.28–2.04) 1.51 (1.05–2.11)
		♀ 2 weeks 2 weeks–1 year 1– 6 years 6–10 years 10–16 years			1.61 (0.94–2.13) 1.79 (1.18–2.47) 1.65 (1.16–2.26) 1.49 (1.19–2.22) 1.41 (0.97–2.23)

* The reference range was taken as the interval between ($\bar{x}$ – 1s) and ($\bar{x}$ + 2s).

The results of *Klett*, quoted in *Schönberg* (13), must also be evaluated critically, since a normal *Gaussian* distribution cannot be assumed for such a small number of probands, and it is therefore invalid to calculate the reference range as $\bar{x}$ ± 1 s.

The same criticism applies to the work of *Borkenstein* et al. (8), which was not primarily concerned with the establishment of reference ranges, but with the description of the normal course of the serum thyroliberin concentration in childhood. The concentrations given in tables 9, 10 and 11 are the basal values of the test. Although the authors stated that their distribution of concentrations displayed a skew to the right, they calculated the mean value and standard deviation for each group, then gave the normal range as the interval of ($\bar{x}$ – 1 s) to ($\bar{x}$ + 2 s). Since they found no significant differences between the selected age groups, they combined all groups and quoted a common reference range for the age range 2 months to 14 years. In view of the small number of probands in each age group, the absence of a normal distribution, and the F-test that was used, this method of proceding must be viewed critically.

Tab. 11. Reference ranges reported in the literature for the serum concentration of thyroxine

Author	Method	Age group	No. of probands	Type of distribution and chosen scatter range	Reference range
Liappis et al., 1988 (11)	LEIA (μg/l)	1–2 days	34	No clear data	182.1 ± 37.7 178.5 106.6 – 257.5
		3–30 days	67		137.8 ± 29.7 135 78.4 – 197.2
		1–12 months	56	$\bar{x}$ ± 1s Median value 95% Scatter range	95.8 ± 21.0 95.0 53.9 – 137.8
		1–7 years	68		87.9 ± 17.7 90.0 52.5 – 123.4
		7–13 years	77		85.5 ± 12.8 86 59.9 – 111.1
		13–18 years	41		77.9 ± 14.3 77.0 49.2 – 106.6
Liappis et al., 1991 (12)	LEIA (μg/l)	1–2 days	28	No clear data	180.5 ± 32.1 178.0 168.0 – 192.9
		3–30 days	48		158.0 ± 40.3 164.5 146.3 – 169.7
		1–12 months	37	$\bar{x}$ ± 1s Median value 95% Scatter range	95.0 ± 16.7 96.0 89.4 – 100.6
		1–7 days	65		89.7 ± 17.1 89.0 85.5 – 94.0
		7–13 years	76		84.6 ± 14.1 83.0 81.4 – 87.9
		13–18 years	42		82.7 ± 13.0 85.5 78.7 – 86.8
Schönberg & Klett, 1980 in *Schönberg*, 1990 (13)	RIA (μg/l)	0.5– 1 month 1.5– 2 months 2.5– 3 months 3.5– 4 months 4.5– 5 months 5.5– 7 months 8.0–12 months	25 19 12 11 11 7 7	No data Mean value ± 1s	111.1 ± 29.5 94.7 ± 27.1 92.6 ± 21.9 97.9 ± 18.9 88.4 ± 24.8 88.2 ± 26.2 75.6 ± 6.5
Borkenstein et al., 1980 (8)	RIA (μg/l)	2 months–14 years	182	No normal distribution Mean value ± 1s	80.5 ± 20.1 (60.4 – 120.7)*
Struckmeyer & Haid, 1986 (9)	RIA (μg/l)	♂ 1–3 days 1 week–1 month 1 month–1 year 1–6 years 6–10 years		No clear data No normal distribution Median value 90% Scatter range	152.0 (105.5–194.7) 121.0 (89.2–186.5) 97.0 (74.0–145.0) 83.0 (63.9–119.0) 90.0 (79.0–118.5)

Continued p. 286

Tab. 11. Continued

Author	Method	Age group	No. of probands	Type of distribution and chosen scatter range	Reference range
		10–16 years		(5% to 95% quantile)	87.0 (77.5–114.5)
		♀ 1–3 days			152.0 (105.5–194.7)
		1 week–1 month			121.0 (89.2–186.4)
		1 month–1 year			108.0 (71.4–129.7)
		1–6 years			9.20 (59.0–108.0)
		6–10 years			91.0 (63.5–116.0)
		10–16 years			85.0 (66.3–115.5)

* The reference range was taken as the interval between ($\bar{x}$ − 1s) and ($\bar{x}$ + 2s).

Tab. 12. Reference ranges reported in the literature for the serum concentration of free thyroxine

Author	Method	Age group	No. of probands	Type of distribution and chosen scatter range	Reference range
Liappis et al., 1987 (10)	RIA (ng/l)	1–2 days	12	No clear data	18.08 ± 2.38 17.75 16.57 – 19.60
		3–30 days	59		17.90 ± 19.60 18.00 17.18 – 18.62
		1–12 months	50	$\bar{x}$ ± 1s Median value 95% Scatter range	15.82 ± 2.37 15.28 15.15 – 16.49
		1–7 years	79		16.01 ± 1.70 15.80 15.63 – 16.39
		7–13 years	90		16.67 ± 2.17 16.24 16.22 – 17.13
		13–18 years	51		16.79 ± 2.66 15.70 16.04 – 17.53
Liappis et al., 1991 (12)	LEIA (ng/l)	1–2 days	28	No clear data	26.17 ± 5.85 25.20 16.4 – 37.87
		3–30 days	48		22.28 ± 3.62 22.55 15.04 – 29.52
		1–12 months	37	$\bar{x}$ ± 1s Median value 95% Scatter range	14.22 ± 1.86 13.80 10.50 – 17.94
		1–7 years	65		12.09 ± 1.91 13.00 9.27 – 16.91
		7–13 years	76		13.38 ± 1.95 13.10 9.48 – 17.28

Tab. 12. Continued

Author	Method	Age group	No. of probands	Type of distribution and chosen scatter range	Reference range
		13–18 years	42		13.68 ± 2.37 13.90 8.94 – 18.42
Schönberg & Klett, 1980 in *Schönberg*, 1990 (13)	RIA (ng/l)	0.5– 1 month	25	No data	23.4 ± 4.9
		1.5– 2 months	19		19.6 ± 5.5
		2.5– 3 months	12	Median value ± 1s	19.2 ± 4.6
		3.5– 4 months	11		20.2 ± 5.0
		4.5– 5 months	11		17.6 ± 6.7
		5.5– 7 months	7		19.2 ± 5.4
		8.0–12 months	7		15.0 ± 4.8

In the present study, we followed the recommendations for hypothyreosis screening put forward by the working group for paediatric endocrinology (13), i. e. that the serum thyrotropin concentration of neonates be determined on the fifth day of life. Table 9 shows the results of an earlier study (14), in which the serum thyrotropin of 1712 healthy neonates was determined under the same conditions with the same test on the fifth day of life. The median value is somewhat lower than that reported in the present work, and the 95% scatter range is somewhat wider. According to the *Mann & Whitney* test, the slight difference between the two random samples is not significant ($p = 0.095$), with an error probability of $\alpha = 0.05$. In cases of suspected congenital hypothyreosis, serum thyrotropin concentrations greater than 10 mU/l should be checked by a control analysis on the following day.

The other authors mentioned in tables 9 to 12 investigated neonates both earlier and later than 5 days post partum. At the same time, they selected wide age intervals in these early years, during which relatively large age-dependent alterations in the concentration of this hormone are to be expected.

With respect to the effect of age on the serum concentrations of all hormones, there is general agreement between the results reported in tables 9 to 12 and the present results.

In all the investigations, the serum thyrotropin concentration in neonates was markedly higher than in all other age groups. Thereafter, the serum thyrotropin concentration showed a tendency to decrease slightly with increasing age. This trend was also confirmed by a correlation analysis.

Tab. 13. Reference ranges for thyrotropin, triidothyronine, thyroxine and free thyroxine in neonates, infants, children and adolescents

Thyrotropin		
Males:	5 days	0.51–7.90 mU/l
	2 months–15 years	0.41–3.45 mU/l
	16–18 years	0.28–1.88 mU/l
Females:	5 days	0.51–7.90 mU/l
	2 months–9 years	0.41–3.45 mU/l
	10–13 years	0.64–4.76 mU/l
	14–15 years	0.41–3.45 mU/l
	16–18 years	0.53–2.85 mU/l
Triiodothyronine		
Males:	5 days	0.99–3.04 µg/l
	2 months–3 years	1.30–3.23 µg/l
	4–15 years	1.50–2.73 µg/l
	16–18 years	1.16–2.35 µg/l
Females:	5 days	0.99–3.04 µg/l
	2 months–3 years	1.3 –3.23 µg/l
	4–13 years	1.50–2.70 µg/l
	14–18 years	1.16–2.35 µg/l
Thyroxine		
Males:	5 days	81.3–233 mg/l
	2–12 months	66.5–158 mg/l
	2– 6 years	55.1–113 mg/l
	7– 9 years	54.3–130 mg/l
	10–13 years	55.1–113 mg/l
	14–18 years	42.3– 99.1 mg/l
Females:	5 days	81.3–233 mg/l
	2 months–3 years	66.5–158 mg/l
	4– 6 years	55.1–113 mg/l
	7– 9 years	54.3–130 mg/l
	10–13 years	55.1–113 mg/l
	14–18 years	42.3– 99.1 mg/l
Free thyroxine		
	5 days	13.0–32.9 ng/l
	2 months–11 years	10.7–17.5 ng/l
	12–18 years	9.2–15.9 ng/l

An unequivocal age-dependent change in the serum concentration of triiodothyronine is not evident from the collected results of other authors, although there is a trend to lower values with increasing age. In the present study, serum triiodothyronine was significantly lower in neonates 5 days post partum than in infants and children. A correlation analysis including all age groups, however, showed a significant negative correlation between age and hormone concentration, i.e. the serum triodothyronine concentration decreased with increasing age.

With the exception of *Borkenstein* et al. (8), all the other authors found age-related changes in serum thyroxine. An early marked decrease of serum thyroxine in neonates is followed by a further downward trend in children and adolescents. In the present study, this downward trend was confirmed by a correlation analysis between age and serum thyroxine concentration.

The results of other authors consistently show that serum concentrations of free thyroxine are markedly higher in neonates than in all other age groups. No further apparent age-dependent changes were noted with increasing age. In the present study, correlation analysis showed a significant negative correlation between age and free thyroxine, i.e. the serum concentration of free thyroxine decreased with increasing age. Statistically, this result is very probably influenced by the high concentrations of serum free thyroxine in neonates 5 days post partum. No significant sex differences were found in any age group.

Table 13 shows the normal ranges for each hormone, as determined in the present study.

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References

1. Deam, D., Goodwin, M. & Ratnaike, S. (1991) Comparison of four methods for free thyroxin. Clin. Chem. *37*, 569–572.
2. Harkavy, K. L. & Enecio, C. E. (1991) Free thyroxine levels in hospitalized newborns: Depressed levels in critical, nonthyroidal illness. J. Perinatol. *XI*, 117–121.
3. Kaptein, E. M., Macintyre, S. S., Weiner, J. M., Spencer, C. A. & Nicoloff, J. T. (1981) Free Thyroxine estimates in nonthyroidal illness: Comparison of eighth methods. J. Clin. Endocrinol. Metab. *52*, 1073–1077.
4. Midgley, J. E. M., Sheehan, C. P., Christofides, N. D., Fry, J. E., Browning, D. & Mardell, R. (1990) Concentrations of free thyroxin and albumin in serum in severe nonthyroidal illness: Assay artefacts and physiological influences. Clin. Chem. *36*, 765–771.
5. Roti, E., Gardini, E., Minelli, R., Bianconi, L. & Flisi, M. (1991) Thyroid function evaluation by different commercially available free thyroid hormone measurement kits in term pregnant women and their newborns. J. Endocrinol. Invest. *14*, 1–9.
6. Witt, J. & Trendelenburg, C. (1982) Gemeinsame Studie zur Erstellung von Richtwerten klinisch-chemischer Kenngrößen im Kindesalter. J. Clin. Chem. Clin. Biochem. *20*, 235–242.
7. International Federation of Clinical Chemistry Scientific Committee, Clinical Section Expert Panel on Theory of Reference Values and International Committee for Standardisation in Haematology. Standing Comittee on Reference Values (1987) Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J. Clin. Chem. Clin. Biochem. *25*, 645–656.
8. Borkenstein, M., Stöffler, G., Stögmann, W., Fueger, G. & Falk, W. (1980) Normwerte für zirkulierende Schilddrüsenhormone, T_3-Uptake und Thyrotropin vor und nach TRH-Gabe. Monatsschr. Kinderheilkd. *128*, 422–427.
9. Struckmeyer, H. & Haid, H. (1986) Richtwerte für das kinderärztliche Laboratorium. 1. Auflage Marburg. Die Medizinische Verlagsgesellschaft.
10. Liappis, N. & Starke, A. (1987) Referenzwerte für die Konzentration von freiem Thyroxin und freiem Trijodthyronin im Serum euthyreoter Kinder. Klin. Pädiatr. *199*, 366–369.
11. Liappis, N. & Schlebusch, H. (1988) Referenzwerte für die Konzentration von Trijodthyronin, Thyroxin und Thyrotropin im Blutserum euthyreoter Kinder. Methode: Lumineszenz-verstärkter Enzymimmunoassay. Klin. Pädiatr. *200*, 410–413.
12. Liappis, N., Schlebusch, H., Von Perjes, M. & Berg, I. (1991) Referenzwerte für die Konzentration des freien Thyroxins, des freien Trijodthyronins und des Thyroxin-bindenden Globulins im Blutserum euthyreoter Kinder. Methode: Lumineszenz-verstärkter Enzymimmunoassay. Klin. Pädiatr. *203*, 113–115.
13. Schönberg, D. (1990) Hyopthyreose-Screening. Endokrinologische Einzelschriften *3*, 27–40.
14. Wiedemann, G. & Jonetz-Mentzel, L. (1992) Reference ranges for thyrotropin in the serum of full-term neonates – compared with the ranges for full-term neonates with various post-partal adaption disorders, and premature neonates. Eur. J. Clin. Chem. Clin. Biochem. *31*, 35–39.

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